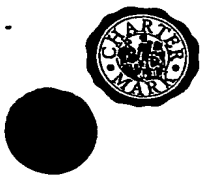

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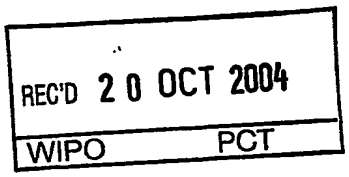

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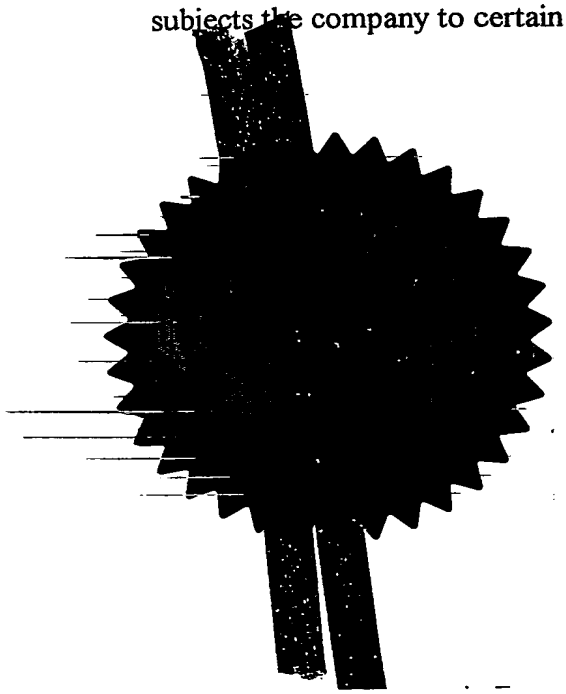

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2.	Patent application number *(The Patent Office will fill in this part)*	19 SEP 2003 0321996.1
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)* 07143563003 Patent ADP number *(if you know it)* If the applicant is a corporate body, give the country/state of its incorporation	NOVARTIS NUTRITION AG LICHTSTRASSE 35 MONBIJOUSTRASSE 118 4056 BASEL 3007 BERN SWITZERLAND SWITZERLAND. SWITZERLAND
4.	Title of invention	Organic compounds
5.	Name of your agent *(If you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* Patents ADP number *(if you know it)*	Craig McLean Novartis Pharmaceuticals UK Limited Patents and Trademarks Wimblehurst Road Horsham, West Sussex RH12 5AB 07181522002 ✓

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Craig McLean 19th September 2003

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Organic Compounds

The present invention concerns the use of saccharides, e.g. oligosaccharides, as inhibitors of pathogen adhesion to mammalian cells, especially of mammalian gut cells, nutritional compositions comprising a saccharide, e.g. oligosaccharide, for the inhibition of pathogen adhesion to mammalian cells and a method for the screening of a saccharide, e.g. oligosaccharide, useful as inhibitor of pathogen adhesion to mammalian cells.

For many gut bacteria, the first stage in pathogenicity is adherence to the gut wall. For this to take place, the bacteria initially attach to particular receptor molecules on the epithelial cell surface, which is mediated by specific carbohydrate groups on the epithelial cell. According to the present invention it has been surprisingly found that saccharides, e.g. oligosaccharides, which mimic these groups can competitively inhibit bacterial binding and reduce the incidence and severity of disease.

In particular, present inventors have identified compounds which survive passage through the gastrointestinal tract and inhibit the adhesion of specific pathogens to the colonic epithelium without adversely affecting the colonic microflora or adhesion of probiotic organisms.

In one aspect of the present invention it has now surprisingly been found that compounds chosen from at least one of manno-oligosaccharide, e.g. alpha 1-2 mannobiose, alpha 1-3 mannobiose, alpha 1-6 mannobiose; methyl manno-oligosaccharide, e.g. methyl alpha manno-oligosaccharide; caseinoglycomacropeptide (CGMP); chito-oligosaccharide; fructo-oligosaccharide (FOS); pectic oligosaccharide; galacto-oligosaccharide (GOS); curdlan (beta-1,3-glucan); sialyl-oligosaccharide; lactose; lactulose; lactosucrose; isomalto-oligosaccharide; oligogalacturonide; partially hydrolysed guar gum; xylo-oligosaccharide; gentio-oligosaccharide; arabino-oligosaccharide and long-chain isomalto-oligosaccharide, hereinafter referred to as "compounds of the invention", show strong antiadhesive activity.

In one embodiment of the invention, the preferred compounds of the invention may comprise manno-oligosaccharide, e.g. alpha 1-2 mannobiose, alpha 1-3 mannobiose, alpha 1-6 mannobiose; methyl manno-oligosaccharide, e.g. methyl alpha manno-oligosaccharide, CGMP; chito-oligosaccharide; pectic oligosaccharide; curdlan; sialyl-oligosaccharide;

lactose; lactulose; lactosucrose; isomalto-oligosaccharide; oligogalacturonide; partially hydrolysed guar gum; xylo-oligosaccharide; gentio-oligosaccharide; arabino-oligosaccharide; long-chain isomalto-oligosaccharide; or mixture thereof.

In another embodiment of the invention, the compounds of the invention may comprise manno-oligosaccharide, e.g. alpha 1-2 mannobiose, alpha 1-3 mannobiose, alpha 1-6 mannobiose; methyl manno-oligosaccharide, e.g. methyl alpha manno-oligosaccharide; pectic oligosaccharide; sialyl-oligosaccharide; chito-oligosaccharide; CGMP; GOS; partially hydrolysed guar gum; xylo-oligosaccharide; lactulose; or mixture thereof.

In yet another embodiment of the invention, the compounds of the invention may comprise manno-oligosaccharide, e.g. alpha 1-2 mannobiose, alpha 1-3 mannobiose, alpha 1-6 mannobiose; methyl manno-oligosaccharide, e.g. methyl alpha manno-oligosaccharide; pectic oligosaccharide; sialyl-oligosaccharide; chito-oligosaccharide; CGMP; or mixture thereof.

In a further aspect the present invention pertains to the use of a composition comprising a compound of the invention for the manufacture of a nutritional or pharmaceutical composition for the inhibition of pathogen adhesion to mammalian cells, e.g. gut mammalian cells, and/or for reducing or inhibiting the invasion and infection of mammalian cells, e.g. gut mammalian cells, by pathogen.

In one embodiment of the invention, the composition of the invention may be used for the treatment of acute or chronic bacteria-associated enteric disorders in a mammal.

In another embodiment of the invention, there is provided a antibacterial and/or virucide composition comprising a compound of the invention.

In yet a further aspect the present invention provides a screening method, e.g. adhesion assay, to test the anti-adhesive activity of a saccharide, e.g. an oligosaccharide, which method comprises

a) adding saccharide, e.g. oligosaccharide, solution to cell monolayers of the human colonic cell line HT29 grown to 90%+, e.g. 95 to 100% confluence, in triplicate wells,

b) adding an equal volume of bacterial culture, e.g. grown in tissue culture medium anaerobically at 37°C, e.g. exponential phase culture, e.g. diluted in phosphate buffered saline,

c) washing of the cell layers after 2h at 37°C aerobic, 5% CO_2, e.g. with phosphate buffered saline (PBS),

d) detaching cell layers e.g. with trypsin/EDTA solution,

e) enumerating bacteria e.g. by plate counting, and

f) comparing counts in wells with saccharides, e.g. oligosaccharides, to those without.

According to the invention the screening method may further comprise

g) performing dose response curves for saccharides, e.g. oligosaccharides, showing antiadhesive activity, and/or

h) determining the effect of the saccharide, e.g. oligosaccharide, on other strains of the pathogen, and/or

i) determining the effect on beneficial bacteria, e.g. probiotic.

In a further aspect of the invention the bacteria culture preparation may include the preparation of subcultures on 3 successive days.

The screening method of the invention is reliable and reproducible and allows a high number of bacteria adhered to the cell monolayer.

Manno-oligosaccharides may be commercially available under the trade name BioMos. Chito-oligosaccharide may be commercially available from Primex Ingredients AS or France Chitine. Sialyl-oligosaccharide may be commercially available from Sunsial E, Taiyo Kagaku Co; LTD. Fructo-oligosaccharide may be commercially available under the trade name Raftilose or Actilite: Galacto-oligosaccharides may be commercially available under the trade name Vivinal GOS, Elixor GOS or Oligomate. Partially hydrolysed guar gum may be commercially available under the trade name Benefiber® from Novartis Nutrition Corporation. Isomalto-oligosaccharide may be commercially available under the trade name Isomalto 900 from Showa Sangyo Co. Oligogalacturonide may be commercially available under the trade name Galursan. Xylo-oligosaccharide may be commercially available under the trade name Xylo-oligo 20P or 35P or 95P from Cuntory Limited. Curdlan (beta,1,3-glucan) may be commercially available from WAKO-Pure-Chemicals-Industries-LTD.

Caseino-oligosaccharide may be commercially available from Arla Foods. Pectic oligosaccharide may be commercially from *Oranges*. Gentio-oligosaccharide may be commercially available.

Alpha 1-6 mannobiose, alpha 1-2 mannobiose and alpha 1-3 mannobiose may be obtained enzymatically from mannose.
To obtain alpha 1-6 mannobiose, the fungal strain *Aspergillus phoenicis*, e.g. *A. phoenicis* ATCC 14332, may be grown in mineral medium, e.g. at pH 5, with BioMOS (Alltech, UK) as sole carbon source, e.g. with 1% BioMOS, under agitation, e.g. on orbital shaker. The incubation condition may be e.g. 30°C, during several days, e.g. 3 days. The inoculation may be done at 10E5 spores/ml. Yield may be about 25%. Enzyme products may then be concentrated, e.g. by ultrafiltration, e.g. 10000 MW cut off, of a sterile filtrate of the incubation medium. The alpha 1-6 mannobiose may then be separated from the monosaccharides e.g. using a P2 gel filtration column, as known to one skilled in the art.

In order to prepare 1,3-alpha-mannosidase, *A. phoenicis* may be incubated during a longer time with BioMOS, e.g. seven days , which results in a production of a second enzyme able to synthetize both alpha-1,6- and alpha-1,3-mannooligosaccharides. Alpha-1,3-mannooligosaccharides may then be purified by technique readily known to one skilled in the art.

In order to prepare alpha 1-2 mannobiose, Aspergillus oryzae, e.g. PM-1, recombinant, overproducer of Penicillium citrinum alpha 1-2 mannosidase may be incubated in DPY medium, e.g. at pH comprised between 3.5 and 6.0, and comprising mannose, e.g. 70% by weight mannose, based on the total weight of the incubation solution. The incubation condition may be e.g. 55°C, during several days, e.g. 8 days. DPY medium is described in Yoshida et al, 1998, Biosci. Biotechnol. Biochem 62, 309-315, the content of which being hereby incorporated by reference.

The pectic oligosaccharides may be obtained through the process described in Olano-Martin E, Mountzouris KC, Gibson GR & Rastall RA (2001), "Continuous production of pectic oligosaccharides in an enzyme membrane reactor", Journal of Food Science 66, 966-971, the content of which being hereby incorporated by reference.

It will be appreciated that such process is readily known to one skilled in the art.

As used herein the term probiotic refers to microorganisms, e.g. live microorganisms, which beneficially affect the host by improving its intestinal microbial balance, such as lactobacilli and bifidobacteria.

As used herein, the term pathogen refers to non beneficial bacteria, viruses, fungi, monocellular or multicellular parasites, toxins and heavy metal cations, for example E. coli, e.g. verocytotoxic E. coli (VTEC) or enteropathogenic E. coli (EPEC), Staphylococcus aureus, e.g. methicillin-resistant Staphylococcus aureus (MRSA), Clostridium difficile or toxins of Clostridium difficile, Sulphate Reducing bacteria, e.g. Desulfovibrio spp, e.g. Desulfovibrio desulfuricans or Desulfovibrio piger.

The screening method of the invention will now be described in more detail.
A culture of the human colonic cells line HT29 is prepared and put into wells, e.g. 12 or 24-well tissue culture plates. The cells may grow to 90% or more confluence, preferably 90 to 100%, more preferably 95 to 100%. The cells may be washed, e.g. with Phosphate Buffer Saline (PBS) buffer, at least once. A bacterial culture is prepared, e.g. Dulbecco's modified eagle medium (DMEM), or in anaerobic tissue culture medium, e.g. Postgate's Medium E , e.g. at 37°C. The bacterial culture may be grown until stationary or logarithmic phase, preferably until exponential phase. The bacterial culture may be diluted 1/500 to 1/10000, more preferably 1/1000, in cell culture medium, e.g. DMEM, or PBS, preferably in PBS, before being added to the cells. The bacterial, e.g. diluted bacterial, culture may be added to the cells in equal volume. The tested oligosaccharide may be added to the bacterial culture, preferably before incubating the culture with the cells, in concentration comprised between about 0.5 to 15 mg/ml, preferably between about 1 and 10 mg/ml, more preferably between about 2 and 5mg/ml, even more preferably at about 2.5mg/ml. The bacterial culture may be incubated with the cells for several hours, preferably from about 1 to about 5 hours, more preferably about 2 hours. The incubation may be done at a temperature comprised between about 35 and about 40°C, preferably at about 37°C. The incubation may be aerobic, e.g. 5% CO_2. The bacteria which don't adhere to the cells may be removed by washing the cells layers, e.g. with PBS, e.g. several times, e.g. three times. The cells may then be removed from the culture plate e.g. by an enzyme/chelator solution, e.g. a trypsin/EDTA solution, e.g. during from about 30 seconds to about 2 minutes. After resuspension, the cells may be

mixed to break up clumps. The adhered bacteria may then be enumerated, e.g. by direct counting e.g. after plating on to plate count agar, or by using an enzyme-based assay, such as beta-galactosidase activity, or a liminus amoebocyte lysate assay. It will be appreciated that such assays are readily known to one skilled in the art, and are available as commercial kits. According to the invention, the bacteria may be stained and the adhered bacteria may be counted with a flow cytometer.

In one aspect of the invention, there is provided a medicament, nutritional or pharmaceutical formulation, for example dietary supplement, comprising a compound of the invention. For the purpose of the invention, the term "composition of the invention" encompasses compositions comprising at least one compound of the invention.

The compositions of the invention may further comprise one or more of the following: proanthocyanidins, lactoferrin, linoleic acid and linolenic acid.

The medicament, nutritional or pharmaceutical composition of the invention may optionally comprise pharmaceutical acceptable carriers. Further, according to the invention there is provided a combined pharmaceutical preparation for simultaneous, separate or sequential use for inhibiting pathogen adhesion to mammalian cells, e.g. for controlling, e.g. treating, preventing or ameliorating acute or chronic bacteria-associated enteric disorders in a mammal.

The compositions of the invention optionally comprise conventional food additives, such as any of emulsifiers, stabilizers, sweeteners, flavourings, colouring agents, preservatives, chelating agents, osmotic agents, buffers or agents for pH adjustment, acidulants, thickeners, texturisers, and so on.

Pharmaceutical compositions and dietary supplements may be provided in the form of soft gel, sachets, powders, syrups, liquid suspensions, emulsions and solutions in convenient dosage forms. In soft capsules the active ingredients are preferably dissolved or suspended in suitable liquids, such as fatty oils, paraffin oil or liquid polyethylene glycols. Optionally stabilisers may be added.

The amount of compound of the invention incorporated into the compositions of the invention may depend on the nature and/or the molecular weight of the saccharide, the form of the compositions of the invention, e.g. a powder or a composition ready-for-consumption. Accordingly, suitable amounts of compound of the invention comprised in compositions according to the invention are in the range of up to about 80 % by weight, e.g. up to about 60% by weight, or up to about 40 % by weight, for example from about 0.05 to about 50% by weight, e.g. from about 0.5 to about 20% by weight, e.g. about 2.5 to about 10%, e.g. about 1% by weight, based on the total weight of the composition.

The amount and dosage regimen of the compositions of the invention to be administered is determined in the light of various relevant factors including the purpose of administration, the age, sex and body weight of individual subject and the severity of the subject's symptoms. When the composition according to the invention is supplied in the form of a food or beverage, a suitable serving size of the compound of the invention may be from about 1mg to about 20g, preferably from about 10 mg to about 10g, more preferably from about 10mg to about 1g. If provided in pharmaceutical form, suitable daily doses of the compound of the invention are up to about 250mg, preferably up to about 150mg, more preferably up to about 100mg, and optimally in the range of about 1mg to about 100mg. In terms of body weight, daily dosage may vary between from about 0.05 mg to about 5g/kg body weight/day, preferably from about 0.5 mg to about 3g/kg body weight/day, more preferably more than 1mg/kg body weight/day, and even more preferably about 1mg/kg body weight/day. The daily dosage may correspond to a single unit dosage, or may be provided through multiple unit dosages. The exact amounts of the compound according to the invention may vary between wide limits and may be readily determined in conventional manner.

Pharmaceutical or dietary supplement forms may be made by conventional compounding procedures known in the pharmaceutical art, that is, by mixing the active substances together with edible pharmaceutically acceptable solid or liquid carriers and/or excipients, e.g. fillers such as cellulose, lactose, sucrose, mannitol, sorbitol, and calcium phosphates and binders, such as starch, gelatin, tragacanth, methylcellulose and/or polyvinylpyrrolidone (PVP). Optional additives include lubricants and flow conditioners, e.g. silicic acid, silicon dioxide, talc, stearic acid, magnesium/calcium stearates, polyethylene glycol (PEG) diluents, disintegrating agents, e.g. starch, carboxymethyl starch, cross-linked PVP, agar, alginic acid and alginates, colouring agents, flavouring agents, and melting agents. Dyes or pigments

may be added to the tablets or dragée coatings, for example for identification purposes or to indicate different doses of active ingredient.

Optionally, the compositions according to the invention may be nutritionally complete, i.e. may include vitamins, minerals, trace elements as well as nitrogen, carbohydrate and fatty acid sources so that they may be used as the sole source of nutrition supplying essentially all the required daily amounts of vitamins, minerals, carbohydrates, fatty acids, proteins and the like. Accordingly, the compositions of the invention may be provided in the form of a nutritionally balanced complete meal, e.g. suited for oral or tube feeding.

Alternatively, the compositions of the invention may be provided as part of a meal, i.e. a nutritional supplement, e.g. in the form of a health drink.

It may be desirable to provide the composition of the invention in the form of a low calorie meal replacement or other nutritional product. In this case the meal replacement or other nutritional product is preferably low fat, i.e. less than about 10 en%, or substantially fat-free, i.e. less than about 2.5 en% contributed by fat, such as about 2 en% fat, based on the total caloric content of the composition. Suitably, a single serving of a low calorie meal replacement will have a caloric value of less than about 1000kcal, and preferably between about 200kcal and about 500kcal.

Suitable compositions of the invention, e.g. suitable low calorie nutritional product, may include soft drink, such as juice, smoothie or soy-based drink, or dispersed in foods of any sort, such as, dairy bars, soups, breakfast cereals, müesli, candies, tabs, cookies, biscuits, spreads, infant formula, weaning food, confectionery, cakes, crackers, such as a rice crackers, and dairy products, such as milk-shake, yoghurt drink, fermented milk.

The compositions of the invention optionally comprise conventional food additives, such as any of emulsifiers, stabilizers, sweeteners, flavourings, colouring agents, preservatives, chelating agents, osmotic agents, buffers or agents for pH adjustment, acidulants, thickeners, texturisers, and so on.

In a further aspect of the invention, there is provided a use of compound or compositions of the invention as food additive.

Suitable product formats according to the present invention include solution, ready-for-consumption composition, e.g. ready-to-drink compositions, instant drink, liquid comestibles, like soft drinks, juice, sports drinks, milk drinks, milk-shakes, yogurt drinks or soup. In a further embodiment of the invention, the compositions of the present invention may be manufactured and sold in the form of a concentrate, a powder, or granules, e.g. effervescent granules, which are diluted with water or other liquid, such as milk or fruit juice, to yield a ready-for-consumption composition, e.g. ready-to-drink compositions or instant drink.

The composition of the invention may be in any form suitable for human administration, and in particular for administration in any part of the gastrointestinal tract. Enteral administration of the compositions of the invention, and preferably oral administration, and administration through a tube or catheter, are covered by the present invention.

The compositions of the invention may be administered under the supervision of a medical specialist, or may be self-administered.

Pharmaceutical, food or beverage incorporating compound according to the invention can be safely-consumed by anyone, and are especially recommended for anyone perceived to be at risk from diseases, conditions and symptoms related to Inflammatory bowel disease (IBD), in particular Ulcerative Colitis and Crohn's disease, colon cancer, Inflammatory bowel disease (IBS), acute or chronic bacteria-associated enteric disorders, e.g. infection of the gastrointestinal tract.

In one embodiment of the invention, the invention pertains to a method of treating and/or preventing e.g. acute or chronic bacteria-associated enteric disorders, IBD, IBS and/or damages of the cells of the gastrointestinal tract caused by toxins or heavy-metal cations, in a mammal, including human, in need of such a treatment, comprising administering to said mammal an effective amount of a compound or composition according to the invention. As used herein, the term "an effective amount" refers to an amount effective to achieve a desired therapeutic effect, such as treating and/or preventing acute or chronic bacteria-associated enteric disorders and/or infection of the gastrointestinal tract.

In another embodiment of the invention, there is provided a method for inhibiting pathogen adhesion to mammalian cells, e.g. to gut mammalian cells.

In a further embodiment, the present invention relates to a process for the production of the compositions of the invention, wherein such process comprises intimately admixing the components of the composition of the invention with pharmaceutically or nutritionally acceptable excipients. Such processes are well known to one skilled in the art.

The utility of all the compositions of the present invention may be observed in standard clinical tests in, for example, indications as described hereinabove, for example using nutritional compositions as described in the Examples hereinbelow, for example using one or more compound of the invention, in a range of from about 1g to 15g, e.g. about 10 g, for a mammal, e.g. adult and in standard animal models. The relief in symptoms characterizing acute or chronic bacteria-associated enteric disorders provided by the compositions may be observed in standard animal tests and in clinical trials, e.g. as monitored by any of the methods known to one skilled in the art, e.g. by analyzing the feacal microflora, e.g. Desulfobrio, or Sulphate reducing bacteria.

One human clinical trial may be affected as follows:
A randomized blind, placebo controlled, parallel study in e.g. 100 subjects may be performed using the composition of the invention. The subjects may receive several times, e.g. three times, a composition of the invention comprising FOS, e.g. in a range of about 6 g/day. Faecal samples may be collected at baseline, after 14 days of treatment and after 28 days of treatment, and faecal bacteria, e.g. Clostridia, may be counted by Fluorescent in situ Hybridisation (FISH), e.g. employing oligonucleotide probes targeting 16S rRNA.

The invention is now further illustrated by the following examples:

Example 1:

Inhibition of *E. coli* Adhesion

VTEC NCTC 12900 was inoculated from frozen stocks into Dulbecco's modified eagle medium (DMEM) with added non-essential amino acids and foetal bovine serum (5% v/v). The culture was incubated anaerobically at 37°C for 18 h. A 20 µl aliquot was transferred

into 2 ml of fresh DMEM, and incubated under the same conditions for 24 h. This was repeated twice more, so that the organism was subcultured on three successive days. An adhesion assay was then carried out as described previously, using 2.5 mg ml methyl-α-D-mannopyrannoside and D-mannose, and the adhered bacteria were enumerated by using

The results indicated good inhibition of adhesion with both methyl-α-D-mannopyrannoside and D-mannose (Figure 1).

Alpha 1,2 mannobiose and alpha 1,6 mannobiose were assayed for anti-adhesive activity using the method described hereinabove, with the modification that the VTEC cultures were subcultured on three successive days as described above. Inhibition of adhesion was detected for both mannobiose sugars and the control (Figure 2).

Further oligosaccharides have been tested for anti-adhesive activity against E. coli, both VTEC and enteropathogenic (EPEC) strains. Methods were as described previously but with three subcultures included in the culture preparation stage, as described above. Compounds tested included Elixor GOS and pectic oligosaccharides that had been further purified by ultrafiltration to remove nitrates. The pectic oligosaccharides showed powerful anti-adhesive activity against both VTEC and EPEC (Figure 3).

Figure 1. Inhibition of adhesion of VTEC to HT29 cells by methyl-alpha-D-mannopyranoside and D-mannose, indicating that adhesion is mediated by type I fimbriae. Error bars indicate standard error. Numbers of adhered bacteria are expressed as a percentage of the numbers adhered in the oligosaccharide-free control in each experiment.

Figure 2. Inhibition of adhesion of VTEC to HT29 cells by methyl-alpha-D-mannopyranoside, alpha -1,6- and alpha -1,2-mannobiose. Error bars indicate standard error.

Figure 3. Anti-adhesive activity of selected saccharides against VTEC and EPEC.

Example 2: **Nutritional composition**

A nutritional composition in form of biscuit is prepared with the following ingredients:

FOS powder	10g
Wheat flour	58.9g
Sugar	8g

Vegetable fat	16g
Wheat flakes	6g
Baking powder	1g
Salt	0.1g

Claims

1. Use of a compound chosen from at least one of manno-oligosaccharides, alpha 1-2 mannobioses, alpha 1-3 mannobioses, alpha 1-6 mannobioses, caseinoglycomacropeptides (CGMP), methyl manno-oligosaccharides, chito-oligosaccharides, fructo-oligosaccharides (FOS), pectic oligosaccharides, galacto-oligosaccharides (GOS), curdlan (beta-1,3-glucan), sialyl-oligosaccharides, lactose, lactulose, lactosucrose, isomalto-oligosaccharides, oligogalacturonide, partially hydrolysed guar gum, xylo-oligosaccharides, gentio-oligosaccharides, arabino-oligosaccharides and long-chain isomalto-oligosaccharides for the inhibition of pathogen adhesion to mammalian cells and/or for reducing or inhibiting the invasion and infection of mammalian cells by pathogen.

2. Use according to claim 1 wherein the compound is chosen from at least one of manno-oligosaccharides, alpha 1-2 mannobioses, alpha 1-3 mannobioses, alpha 1-6 mannobioses, methyl manno-oligosaccharides, pectic oligosaccharides, sialyl-oligosaccharides, chito-oligosaccharides, caseinoglycomacropeptide, galacto-oligosaccharides (GOS), partially hydrolysed guar gum, xylo-oligosaccharides and lactulose.

3. A nutritional or pharmaceutical composition comprising a compound chosen from at least one of manno-oligosaccharides, alpha 1-2 mannobioses, alpha 1-3 mannobioses, alpha 1-6 mannobioses, caseinoglycomacropeptides (CGMP), methyl manno-oligosaccharides, chito-oligosaccharides, fructo-oligosaccharides (FOS), pectic oligosaccharides, galacto-oligosaccharides (GOS), curdlan (beta-1,3-glucan), sialyl-oligosaccharides, lactose, lactulose, lactosucrose, isomalto-oligosaccharides, oligogalacturonide, partially hydrolysed guar gum, xylo-oligosaccharides, gentio-oligosaccharides, arabino-oligosaccharides and long-chain isomalto-oligosaccharides and a nutritionally or pharmaceutically acceptable excipient.

4. Use of a compound chosen from at least one of manno-oligosaccharides, alpha 1-2 mannobioses, alpha 1-3 mannobioses, alpha 1-6 mannobioses, caseinoglycomacropeptides (CGMP), methyl manno-oligosaccharides, chito-oligosaccharides, fructo-oligosaccharides (FOS), pectic oligosaccharides, galacto-oligosaccharides (GOS), curdlan (beta-1,3-glucan), sialyl-oligosaccharides, lactose, lactulose, lactosucrose, isomalto-oligosaccharides, oligogalacturonide, partially hydrolysed guar gum, xylo-oligosaccharides, gentio-

oligosaccharides, arabino-oligosaccharides and long-chain isomalto-oligosaccharides for the manufacture of a nutritional or pharmaceutical composition for the inhibition of pathogen adhesion to mammalian cells, and/or for reducing or inhibiting the invasion and infection of mammalian cells by pathogen.

5. Use of a compound chosen from at least one of manno-oligosaccharides, alpha 1-2 mannobioses, alpha 1-3 mannobioses, alpha 1-6 mannobioses, caseinoglycomacropeptides (CGMP), methyl manno-oligosaccharides, chito-oligosaccharides, fructo-oligosaccharides (FOS), pectic oligosaccharides, galacto-oligosaccharides (GOS), curdlan (beta-1,3-glucan), sialyl-oligosaccharides, lactose, lactulose, lactosucrose, isomalto-oligosaccharides, oligogalacturonide, partially hydrolysed guar gum, xylo-oligosaccharides, gentio-oligosaccharides, arabino-oligosaccharides and long-chain isomalto-oligosaccharides for the manufacture of a nutritional or pharmaceutical composition for the treatment of acute or chronic bacteria-associated enteric disorders in a mammal.

6. A screening method to test the anti-adhesive activity of an oligosaccharide which method comprises

a) adding oligosaccharide solution to cell monolayers of the human colonic cell line HT29 in triplicate wells,

b) adding an equal volume of bacterial culture,

c) washing of the cell layers after 2h at 37°C aerobic, 5% CO_2,

d) detaching cell layers with trypsin/EDTA solution,

e) enumerating bacteria by plate counting, and

f) comparing counts in wells with oligosaccharides to those without.

Abstract

The present invention concerns compositions comprising a saccharide for the inhibition of pathogen adhesion to mammalian cells.

Fig. 1

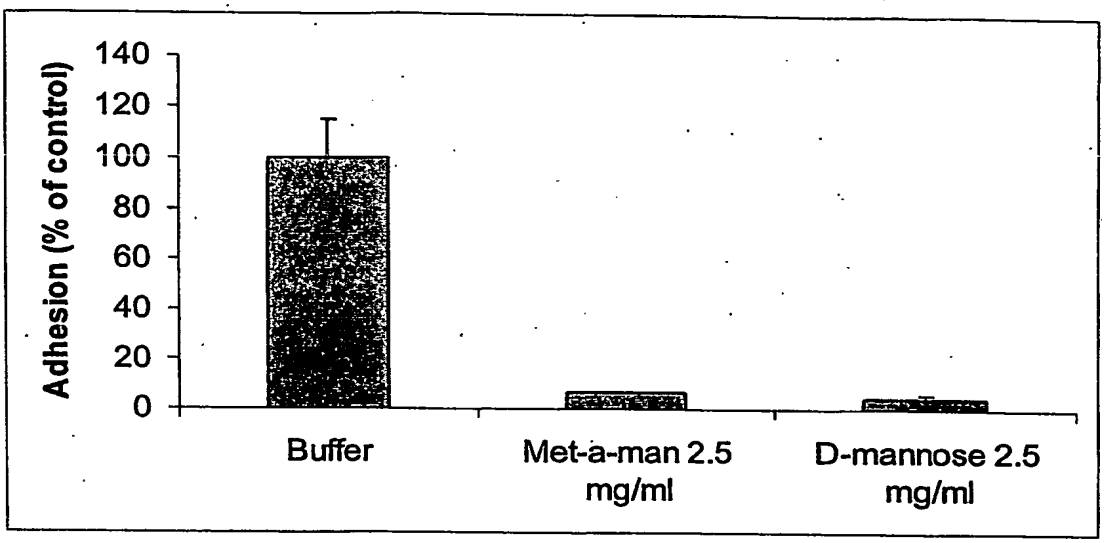

Adhesion (% of control)
140
120
100
80
60
40
20
0
Buffer
Met-a-man 2.5 mg/ml
D-mannose 2.5 mg/ml

Fig. 2

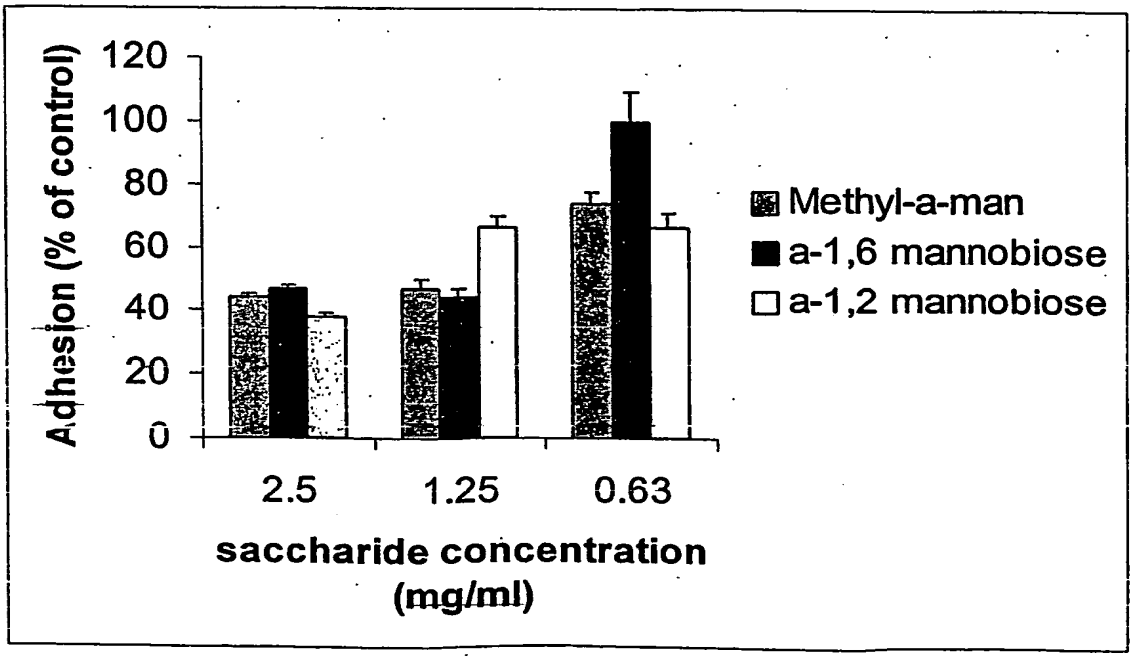
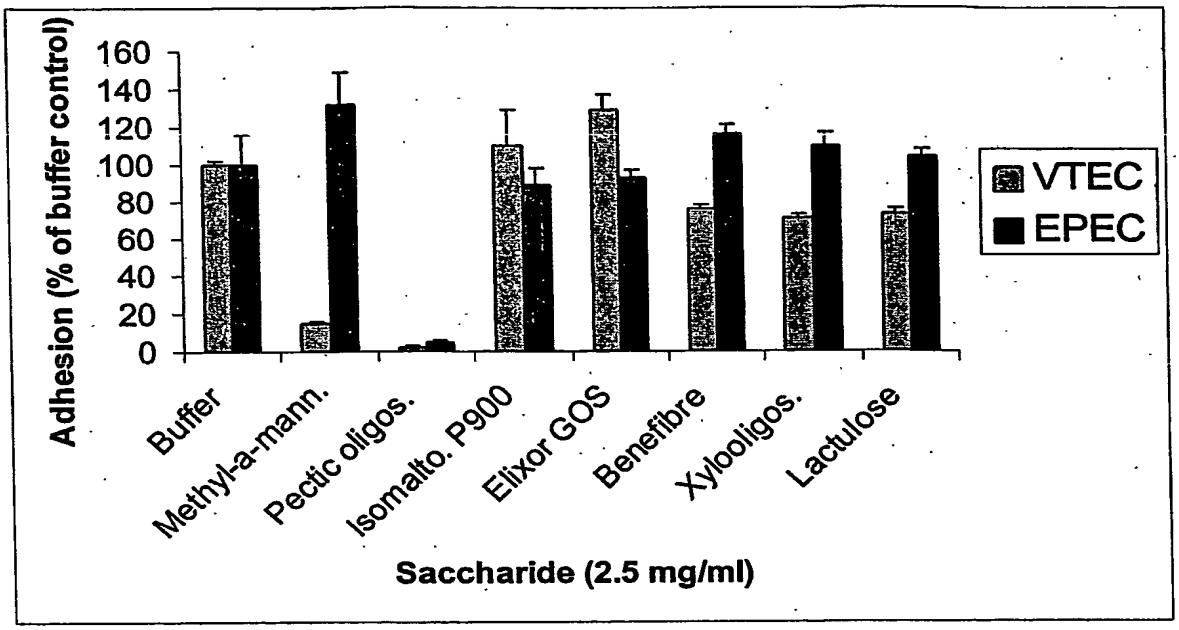

Adhesion (% of control)
120
100
80
60
40
20
0
2.5
1.25
0.63
saccharide concentration (mg/ml)
Methyl-a-man
a-1,6 mannobiose
a-1,2 mannobiose

Fig. 3


Adhesion (% of buffer control)
160
140
120
100
80
60
40
20
0
Buffer
Methyl-a-mann.
Pectic oligos.
Isomalto. P900
Elixor GOS
Benefibre
Xylooligos.
Lactulose
Saccharide (2.5 mg/ml)
VTEC
EPEC